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Mitochondrial ROS Production Contributes to Generation of Ca Waves During Beta-Adrenergic Receptor Stimulation in Rabbit Ventricular Myocytes

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Beta-adrenergic receptor (beta-AR) activation leads to positive inotropic effect but can also increase diastolic sarcoplasmic reticulum (SR) Ca release in the form of pro-arrhythmic Ca waves. Beta-AR activation increases cellular energy demand and mitochondrial reactive oxygen species (ROS) production. We investigated the role of mitochondrial ROS in the generation of Ca waves during beta-AR stimulation. In electrically stimulated myocytes, application of ISO (0.1 microM) led to the occurrence Ca waves during diastole. The frequency of Ca waves increased over time during ISO application, with a particular steep increase after 6 minutes. Frequency of ISO-mediated Ca waves positively correlated with mitochondrial ROS production measured with Mito-SOX (a dye specifically targeted to mitochondria). The mitochondria specific antioxidant Mito-Tempo (5 microM) effectively prevented the ISO-mediated ROS production, as well as ISO-mediated Ca waves. Inhibition of the mitochondrial respiration with rotenone (0.3 microM) produced similar preventive effects on mitochondrial ROS production and Ca wave generation. Measurements of intra-SR free Ca ([Ca]SR) showed an initial increase of [Ca]SR (SR Ca load) followed by a gradual decline over time during ISO application. This decline of [Ca]SR was the result of increased SR Ca leak particularly in the form of Ca waves. Mito-Tempo did not affect the initial increase in SR Ca load, however, the ROS scavenger significantly prevented the [Ca]SR decline in the presence of ISO. Furthermore, Mito-Tempo significantly reduced ISO-mediated augmentation of SR Ca leak. These results suggest that mitochondrial ROS production play an important role in the generation of Ca waves during beta-AR stimulation.

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Calcium-Mediated Arrhythmia Substrates Associated with Oxidative Stress during Myocardial Infarction

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Following myocardial infarction (MI), ventricular arrhythmias commonly originate from the MI border zone (BZ) but the cause for this is not clear. Increased oxidative stress is a hallmark of MI and an important mechanism of intracellular calcium dysregulation. We hypothesize that following MI, increased oxidative stress in the infarct BZ creates a substrate for calcium-mediated arrhythmias. Methods: Male Lewis rats (n=12) underwent ligation of the left-anterior descending artery. At 4 weeks, hearts were isolated and high-resolution optical mapping of intracellular calcium (Indo-1AM) and reactive oxygen species (ROS, dichlorofluorescein diacetate) were performed. Blebbistatin (7μM) was used to eliminate motion artifact. Calcium transient alternans (CaT_Alt) and multicellular-spontaneous calcium release (mSCR) were induced by rapid pacing (200–670 bpm) before and after the CaMKII blocker KN-93. Sites within 2mm of the anatomical scar border where designated as BZ. All other sites in the mapping field outside the BZ and scar were considered remote. Results: CaT_Alt and mSCR activity were significantly greater in the MI BZ ($15\pm3\%$, $20\pm2\%$) as compared to remote sites ($3.3\pm2\%$, $p<0.01$; $9.0\pm2\%$, $p<0.001$), respectively. Additionally, ROS density was increased by $283\pm53\%$ ($p<0.001$) in the BZ compared to remote regions, which was confirmed offline by tissue sample analysis. Interestingly, treatment with KN-93 significantly decreased CaT_Alt in the BZ by $50\pm10\%$ ($p<0.05$) but did not decrease mSCR activity. Conclusions: These results demonstrate that increased ROS in the infarct BZ is associated with a significant increase in calcium-mediated arrhythmia substrates (CaT_Alt and mSCR). In addition, CaMKII activation may be a mechanism of CaT_Alt but not spontaneous calcium release in the BZ, suggesting multiple calcium regulatory targets of oxidative stress associated with MI.

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ROS Dependent Modulation of Calcium Sparks in Cardiomyocytes

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Mitochondrial regulation of cytosolic calcium ($[Ca^{2+}]_i$) is thought to depend on the mitochondrial inner membrane potential ($\Delta\Psi_m$). $\Delta\Psi_m$ arises from activity of the electron transport chain and is thought to play a critical role in all ion movements across the inner membrane. With $\Delta\Psi_{mito} \approx -150$ mV to -200 mV, there is clearly a strong electrochemical potential for the movement of Ca^{2+} from the cytosol (about 100 nM) into the matrix (around 100 nM). If significant rapid Ca^{2+} influx occurs, then $\Delta\Psi_{mito}$ per se should influence the time-course, frequency, magnitude and other characteristics of Ca^{2+} sparks and the $[Ca^{2+}]_i$ transients. Tetramethylrhodamine methyl ester (TMRM) was used to monitor $\Delta\Psi_{mito}$ in freshly isolated rat cardiomyocytes and photon stress was used to depolarize the mitochondria. In addition to the changes in electrochemical potential for Ca^{2+} entry, depolarization of mitochondria was associated with an increase in cellular reactive oxygen species (ROS) as measured by DCF (as has been previously reported by several laboratories). Quantitative analysis of these findings permits us to separate the influence of each on the changes in Ca^{2+} signaling observed. Consistent with findings by Zhou et al., 2011, we report a role for local [ROS] in altering Ca^{2+} signaling.

References

Zhou, L., Aon., M.A., Lui, T., O'Rourke, B. Dynamic modulation of Ca^{2+} sparks by mitochondrial oscillations in isolated guinea pig cardiomyocytes under oxidative stress. *JMCC*. 2011. 51(5):632–9.

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Ryanodine Receptor Use-Dependent Block Suppresses Ca^{2+} Waves in Permeabilized Casq2-/- and RyR2-R4496C Myocytes

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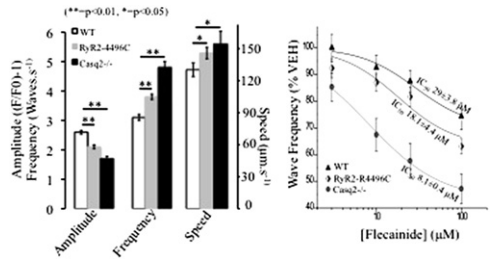
Background-Casq2 and RyR2 mutations can both cause catecholaminergic-polymorphic ventricular tachycardia (CPVT). Leaky RyR2s (increased open probability) generate Ca^{2+} waves, delayed afterdepolarizations and ventricular arrhythmias. We hypothesize that the RyR2 open-state inhibitors flecainide (FLEC) and R-propafenone (RPROP) abolish this sequence of events in both CPVT genotypes.

Methods and Results-Using confocal microscopy, we recorded Ca^{2+} waves in permeabilized myocytes from casq2-/-, RyR2-R4496C and wild-type (WT) mice. Casq2-/- myocytes showed the highest wave frequency and speed and the lowest amplitude, RyR2-R4496C exhibited intermediate values and WT had the lowest values (Figure). We next obtained FLEC and RPROP concentration-response curves and calculated IC_{50} and efficacy of wave inhibition. Both FLEC and RPROP reduced all wave parameters with higher potency (lower IC_{50}) and efficacy in casq2-/- and RyR4496C compared to WT (FLEC: IC_{50} 12μM vs 22μM vs 49μM; efficacy 43% vs 29% vs 21% for casq2-/-, RyR2-R4496C and WT respectively. RPROP: IC_{50} 18μM vs 28μM; efficacy 36% vs 17% for RyR2-R4496C and WT respectively; n=10-60 cells per group).

Conclusion-RyR2 activity determines the potency and efficacy of open-state blockers for suppressing arrhythmogenic Ca^{2+} waves in permeabilized myocytes from casq2-/- and RyR2-R4496C CPVT models.



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Ca^{2+} Wave Velocity in Cardiomyocytes is Regulated by Ryanodine Receptor Ca^{2+} Sensitivity and SR Ca^{2+} Content

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Arrhythmias can be elicited by sudden release of Ca^{2+} from the sarcoplasmic reticulum (SR) via the SR Ca^{2+} release channel (RyR) in cardiomyocytes. Such release may initiate a self-propagating process called a Ca^{2+} wave, which may trigger a spontaneous action potential. We hypothesized that likelihood of arrhythmia is augmented when waves propagate more rapidly, and investigated the role of RyR Ca^{2+} sensitivity and SR Ca^{2+} content. Ca^{2+} waves were studied in isolated ventricular cardiomyocytes from mice using confocal microscopy and Ca^{2+} fluorescence. Waves preceding spontaneous action potentials propagated more rapidly than those that did not generate action potentials ($p<0.05$). Thus, mechanisms controlling wave speed may determine arrythmogenic potential. We investigated effects of increased RyR Ca^{2+} sensitivity by rapidly exposing cells to 1 mM caffeine. The first wave

following the switch to caffeine exhibited increased propagation velocity ($p<0.05$). However, at steady-state, velocity was not altered from pre-treatment values, and mean wave magnitude and SR Ca^{2+} content were reduced ($p<0.05$). The opposite intervention, reducing RyR sensitivity with 100 μM tetracaine, increased wave magnitude and SR Ca^{2+} content at steady state ($p<0.05$) but did not alter wave speed. These observations suggest that SR content-induced alterations in RyR sensitivity could account for differences in wave speed between initial and steady-state conditions. To test this hypothesis, we increased SR content by pacing cells at 5 Hz, and then stopped the stimulation to allow SR content to decline. Wave speed was observed to progressively decrease following termination of stimulation ($p<0.05$). Our data suggest that RyR sensitivity and SR Ca^{2+} content are important determinants of Ca^{2+} wave speed. An induced increase in RyR sensitivity, possibly relevant in heart failure, increases wave speed only until counteracted by steady-state reduction in SR content.

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Self-Organized Criticality Underlies Arrhythmogenic Calcium Waves in Cardiac Myocytes

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In cardiac myocytes, calcium (Ca)-induced Ca release can give rise to propagating Ca waves that promote cardiac arrhythmias. To understand the mechanism underlying the transition from localized Ca sparks to global Ca waves, we developed a mathematical model of a spatially-distributed network of diffusively coupled Ca release units (CRUs) comprised of junctional/longitudinal sarcoplasmic reticulum and dyadic/cytoplasmic spaces with physiologically realistic dimensions. As total Ca was increased in the model, Ca spark cluster sizes initially exhibited an exponential distribution, which transitioned to a scale-free power-law ($f^{-\beta}$) distribution near the threshold at which random Ca sparks transitioned to Ca waves. The power-law relationship indicates that Ca release patterns in the CRU network are governed by a dynamical mechanism called self-organized criticality, the same process underlying many natural world phenomena such as avalanches and earthquakes. We tested this prediction experimentally in saponin-permeabilized cardiac myocytes. As free Ca, buffered with 0.5 mM EGTA, was increased, Ca spark cluster size transitioned from an exponential distribution to a power-law distribution at a free Ca concentration of 400 nM. Consistent with self-organized criticality, this Ca concentration was near the threshold for spark-to-wave transition since fully propagating waves were observed above 400 nM Ca. Below this concentration, Ca was released mostly as individual sparks, and spark cluster sizes followed an exponential rather than power-law distribution. In conclusion, our findings provide both theoretical and experimental evidence that the transition from Ca sparks to arrhythmogenic Ca waves in cardiac myocytes is mediated by the dynamical process of self-organized criticality, common to many natural phenomena. This provides a theoretical framework for developing interventions which modulate the Ca spark-to-wave transition threshold as a potential therapeutic strategy for preventing arrhythmias.

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Can the Sodium-Calcium Exchanger Initiate or Supress Calcium Sparks in Cardiac Myocytes?

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Calcium (Ca) sparks in cardiac myocyte are formed by positive feedback (Ca induced Ca release). At the resting membrane potential (V_m ~-80 mV), the sodium-calcium exchanger (NCX) removes Ca from the cell (forward mode). If Ca released from the sarcoplasmic reticulum (SR) is quickly removed via forward mode NCX before the positive feedback process starts, the Ca release becomes non-spark Ca leak. However, at higher V_m, Ca enters through the NCX (reverse mode) and Ca entry through reverse mode NCX may activate ryanodine receptors (RyRs) and initiate Ca sparks. These are also influenced by the cleft/non-cleft distribution of NCX, which is still unknown. In this study, using physiologically detailed mathematical model of the subcellular Ca cycling, we investigate how the magnitude of NCX current and the distribution of NCXs alter Ca spark formation. We find that at the resting membrane potential if all NCXs are localized to the cleft, Ca sparks are significantly reduced (at $[Ca]_{SR}$=700 μM, there is a 40% reduction vs. the case where all NCXs locate outside of the cleft). During excitation-contraction coupling, most Ca sparks are induced by L-type Ca current (I_{CaL}) and only a small fraction of Ca sparks are due to NCX current at V_m = 0mV. If V_m is higher (+40 mV), since I_{CaL} becomes smaller and NCX becomes larger, more sparks are induced by NCX. But the absolute number of Ca sparks is limited. These results also strongly depend on the distribution of NCXs. If many NCXs locate close to RyRs, NCX currents initiate Ca sparks much more efficiently. This underscores the criticality of NCX localization regarding functional impact on SR Ca release.

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Microscopic Analysis of Calcium Dynamics in Single Migrant Cells in Response to a Heat Pulse

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Ca^{2+} dynamics plays a key role in many fundamental reactions such as muscle contraction. In this study, we focused on two kinds of phenomena regarding Ca^{2+} dynamics. Firstly, we previously revealed that in HeLa cells a rapid elevation of Ca^{2+} concentration (Ca^{2+} burst) can be induced by a heat pulse [Tseeb, V. *et al.*, *HFSP J.* **3**, 117–123 (2009)]. Secondly, Wei *et al.* demonstrated that discrete, local and short-lived high-Ca^{2+} microdomains (Ca^{2+} flickers) determine the direction of the cell migration in WI-38 cells [Wei, C. *et al.*, *Nature* **457**, 901–905 (2009)]. Here, we studied the effects produced by a heat pulse in WI-38 cells. The medium in the vicinity of single WI-38 cells was heated by focusing a 1455-nm IR laser. The effects of the heat pulse on Ca^{2+} dynamics were studied by means of a fluorescence Ca^{2+} indicator, Fluo-4. We found that the heat pulse induced Ca^{2+} burst in WI-38 cells in a similar manner as in HeLa cells. Intracellular Ca^{2+} concentration decreased during heating and rapidly increased at the onset of re-cooling. At the room temperature, Ca^{2+} burst was induced only by large ΔT, whereas at the human body temperature Ca^{2+} burst could be induced by much smaller ΔT. In other words, WI-38 cells are more thermo-sensitive at the human body temperature than at the room temperature. Furthermore, the experiments using intracellular inhibitors suggested that Ca^{2+} pumps, SERCAs, were activated at higher temperature, and Ca^{2+} was released from concentrated ERs to cytosol through IP_3Rs.

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Ca^{2+} Heterogeneity Within a Ca^{2+} Spark

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The Ca^{2+} spark is the elemental Ca^{2+} release event in excitation-contraction coupling. The synchronized summation of thousands (10,000 to 20,000 per cell) of these local Ca^{2+} release events will give rise to the normal Ca^{2+} transient that underlies contraction. Ca^{2+} sparks represent a widely found Ca^{2+} release process among different types of tissue (muscles, neurons and even non-excitable cells) and species (rat, mouse, guinea-pig, rabbit, dog, cat, human) although the details are tissue and species specific. Ca^{2+} sparks are the local events showing the increase of Ca^{2+} in the cytosol following activation of ryanodine receptors (RyR) at the junctional sarcoplasmic reticulum (jSR). This increase of $[Ca^{2+}]$ in the cytosol is matched by a decrease of Ca^{2+} within the jSR (Ca^{2+} blink). Ca^{2+} blinks were detected by using the low affinity Ca^{2+} indicator fluo-5N loaded into the SR. Ca^{2+} blinks are even more localized events (FWHM=1 μM versus 2.2 μM for sparks) than Ca^{2+} sparks, reflecting the inner structure of the SR. Simultaneous visualization of Ca^{2+} sparks and Ca^{2+} blinks has allowed the detection of a new sub-population of Ca^{2+} release events that are smaller than Ca^{2+} sparks and these have been called ''quarky Ca^{2+} release events (QCR)'' (Brochet et al., 2011) because they are intermediate in size between Ca^{2+} release by a single RyR channel and the entire cluster of RyR in the jSR. QCR events have also been hypothesized to be commingled with the dynamics of Ca^{2+} release during a spark. Here, we visualize heterogeneity of Ca^{2+} release within a spark. The importance of this observation and its relationship with the organization of RyR at the jSR will be discussed. These results provide important new understanding of cardiac Ca^{2+} signaling.

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Parameter Sensitivity Analysis of a Stochastic Ca^{2+} Spark Model using Novel Computational Methods

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Parameter sensitivity analysis is useful for identifying how changes in model parameters affect measurable model outputs. Stochastic models, however,